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Recipients Receiving Better HLA-Matched Hematopoietic Cell Transplantation Grafts, Uncovered by a Novel HLA Typing Method, Have Superior Survival: A Retrospective Study

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A B S T R A C T

HLA matching at an allelic-level resolution for volunteer unrelated donor (VUD) hematopoietic cell transplantation (HCT) results in improved survival and fewer post-transplant complications. Limitations in typing technologies used for the hyperpolymorphic HLA genes have meant that variations outside of the antigen recognition domain (ARD) have not been previously characterized in HCT. Our aim was to explore the extent of diversity outside of the ARD and determine the impact of this diversity on transplant outcome. Eight hundred ninety-one VUD-HCT donors and their recipients transplanted for a hematologic malignancy in the United Kingdom were retrospectively HLA typed at an ultra-high resolution (UHR) for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 using next-generation sequencing technology. Matching was determined at full gene level for HLA class I and at a coding DNA sequence level for HLA class II genes. The HLA matching status changed in 29.1% of pairs after UHR HLA typing. The 12/12 UHR HLA matched patients had significantly improved 5-year overall survival when compared with those believed to be 12/12 HLA matches based on their original HLA typing but were found to be mismatched after UHR HLA typing (54.8% versus 30.1%, $P = .022$). Survival was also significantly better in 12/12 UHR HLA-matched patients when compared with those with any degree of mismatch at this level of resolution (55.1% versus 40.1%, $P = .005$). This study shows that better HLA matching, found when typing is done at UHR that includes exons outside of the ARD, introns, and untranslated regions, can significantly improve outcomes for recipients of a VUD-HCT for a hematologic malignancy and should be prospectively performed at donor selection.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) provides a curative treatment option for individuals with hematologic diseases. Recipients and donors are considered to be

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compatible or "well matched" if they share a high degree of genetic similarity at their HLA loci. Historically, HCT was limited to using HLA-identical related donors. Improved knowledge of the transplant process and therapeutic drug development, coupled with increased resolution of HLA typing and a significantly larger pool of volunteer unrelated donors (VUDs) worldwide, has meant that HCT with a VUD now offers comparable survival [1,2]. Current HCT activity shows that the use of VUDs as a source of stem cells is greater than related donors [3,4].

The gold standard for HLA matching between recipients and VUDs has changed little in the last 10 years, with either an 8/8 or 10/10 HLA allelic match (HLA-A, -B, -C, -DRB1, and -DQB1 matched) as the preferred option [5,6]. Matching at HLA-DQB1 has been shown to have significant advantages [6,7], but the benefit of matching has been controversial historically. Although matching for HLA-DPB1 has been shown to be beneficial [8,9], the existence of a recombination hotspot between the HLA-DQB1 and -DPB1 loci means that allelic matching for HLA-DPB1 is challenging [10]. Consequently, several models of permissive HLA-DPB1 mismatching have been proposed [7-9,11,12]. Despite the use of 10/10-matched, HLA-DPB1 permissively (mis)matched donors, the incidence of transplant complications such as graft-versus-host disease (GVHD) and disease relapse remain significant and are associated with worse outcomes [6,13]. Secondary donor factors such as younger donor age [2,6,13] and cytomegalovirus (CMV) matching, either in combination with HLA matching [6] or independently [2], have been correlated with improved outcomes.

Despite data showing the beneficial impact of allelic-level HLA matching [5], limitations in technology and capacity and the hyperpolymorphic nature of HLA genes have often prevented histocompatibility typing laboratories from achieving this level of resolution [14]. Many high-throughput methodologies have focused solely on exons of the gene that encode the antigen recognition domain (ARD) because of the assumption that these functionally relevant regions are the most important regions to match for HCT. Indeed, studies suggest that diversity outside of the ARD is limited in well-matched VUD-HCT pairs [15].

As the number of HLA alleles identified has increased, so has the number of ambiguous combinations of alleles that cannot be resolved by typing strategies that only include the ARD [16,17]. The ideal HLA typing paradigm has always been to provide fully phased sequence-level typing that covered the entire gene [18]. The advent of next-generation sequencing (NGS) and third-generation sequencing technologies has brought this paradigm closer to reality by providing the possibility to characterize full HLA gene sequences in both a research and clinical setting. Although many HLA typing laboratories have moved to using NGS methods to enable refined HLA typing results, the impact of ultra-high resolution (UHR) HLA typing on HCT outcome has not been studied. The aim of this study was to identify for the first time the impact of better matching through HLA typing data at UHR, as achieved by Single Molecule Real-Time DNA sequencing, on the outcome of VUD-HCT.

METHODS

Patient Cohort

The cohort in this study is part of a large retrospective study consisting of VUD–recipient HCT pairs transplanted between 1996 and 2011. The study comprised 891 adult and pediatric patients with hematologic malignancies from 32 UK allogeneic centers (Table 1).

Clinical outcome data were collected as part of a collaboration with the British Society of Blood and Marrow Transplantation. Primary outcomes included overall survival (OS), nonrelapse mortality (NRM), disease relapse, and acute GVHD (aGVHD). Reporting of chronic GVHD follow-up data were insufficient to perform this analysis.

Ethical Approval

Ethical approval for this study was granted from the National Research Ethics Service (application number MREC 01/8/31; www.myresearchproject.org.uk) and is a registered study with the Integrated Research Application System (project ID: 168991). Written consent was sought from all participants before donation or transplant.

HLA Typing

Methods of DNA-based HLA typing used previously included sequence specific oligonucleotide probing, Sanger sequencing-based typing, and reference strand-mediated conformational analysis [19]. Typing strategies mainly included exons that encoded the ARD.

Retrospective UHR HLA typing of the cohort was performed for the 6 classic HLA loci using Pacific Biosciences Single Molecule Real-Time sequencing (Menlo Park, CA, USA), as described previously [18,20,21]. Patients were only eligible for further clinical analyses when complete UHR HLA typing was available for both patient and donor samples. HLA typing results were analyzed blind and subsequently compared with previous typing data. All discrepancies from previous typing were investigated. Matching between donors and recipients was determined at a genomic level for HLA class I loci (ie, definitive allelic matching) and at a coding DNA sequence (CDS) level for HLA class II loci, including all exons that encode the expressed extracellular domains of the mature protein.

Models of HLA Matching

It is generally accepted that 12/12 HLA-matched patients have a better outcome prognosis than those who are less well matched. Therefore, an aim was to compare the outcome of this group of patients after UHR HLA typing once it was known how many pairs remained a 12/12 UHR HLA match and how many that were now found to be mismatched. Additionally, we aimed to show how outcome differed for these 12/12 UHR HLA-matched patients in comparison with any degree of mismatch as identified with this typing strategy. Finally, we aimed to show whether noncoding variation alone or mismatching of non-ARD exons had an impact on patient outcome.

To determine if there were viable alternatives to a 12/12 UHR HLA match, we chose to test the HLA-DPB1 T cell epitope (TCE) model of permissible mismatching in this data set. Additionally, outcome analyses included the combined impact of UHR HLA matching and CMV matching using a model described previously [6]. Here, recipients and donors with the same CMV serostatus were considered to be matched (recipient and donor both CMV seropositive or both CMV seronegative). Any other combination was considered to be CMV mismatched.

Statistical Methods

Probability curves for OS were calculated by the Kaplan-Meier method and compared using the log-rank test. Outcomes with competing events (disease relapse and NRM) were determined using the cumulative incidence function with Gray's test used for comparisons of groups [22]. Proportions of patients with aGVHD (Glucksberg criteria) [23] were compared with the chi-squared test. HLA-matching variables were adjusted for known prognostic variables in multivariate analyses using Cox regression, Fine and Gray, or logistic regression analysis as appropriate. All statistical tests were 2-sided, and significance was determined when $P \leq .05$. Multivariate analyses included variables where univariate analysis outcomes were $P \leq .2$. Analyses were performed using SPSS version 24 (SPSS, Inc., Chicago, IL) or R version 3.4.2 [24].

RESULTS

Impact of UHR 12/12 HLA Matching on HCT Outcome

The previously assigned HLA matching status changed in 29.1% of pairs after UHR HLA typing (Supplementary Table S1). Previously unknown variation was identified in 24.8% of pairs (n = 221), most commonly due to the existence of intron and/or UTR polymorphisms (n = 161; Supplementary Table S2) and/or novel variants that were not detected previously due to limitations of the methodology used or the location of the polymorphism (n = 104).

The 5-year OS of the cohort was 41% (95% confidence interval [CI], 38 to 45). The presence of previously undiscovered mismatch(es) was associated with significantly lower OS compared with those that were matched (5-year OS: 30.1% [95% CI, 14 to 54] versus 54.8% [95% CI, 42 to 67]; $P = .022$) (Figure 1A). Furthermore, UHR 12/12 HLA-matched patients (n = 81) also

Table 1
Study Cohort Characteristics (N = 891)

Variable	12/12 UHR HLA Match(n = 81)	12/12 CDS HLA Match(n = 13)	All Other Mismatches (n = 797)	*P*	All Mismatches (n = 810)	*P* (12/12 UHR HLA Match vs. All Other Mismatches)
Median donor age, yr (range)	33.4 (20.4-53.0)	41.2 (22.6-53.5)	34.8 (19.0-58.9)	.13	34.8 (19.0-58.9)	.054
Median recipient age, yr (range)	43.2 (3.4-70.5)	42.7 (2.7-58.1)	40.8 (1.1-71.9)	.39	40.9 (1.1-71.9)	.22
1-18 yr	9 (11.1)	3 (23.1)	141 (17.7)		144 (17.8)	
>18 yr	72 (88.9)	10 (76.9)	656 (82.3)		666 (82.2)	
Patient age, yr						
<40	36 (44.4)	6 (46.2)	386 (48.4)	.78	392 (48.4)	.50
>40	45 (55.6)	7 (53.8)	411 (51.6)		418 (51.6)	
Donor age, yr						
<30	34 (42.0)	4 (30.8)	236 (29.6)		240 (29.6)	
>30	47 (58.0)	9 (69.2)	553 (69.4)	.08	562 (69.4)	*.02*
Missing	0	0	8 (1.0)		8 (1.0)	
Sex, male						
Recipients	49 (60.5)	6 (46.2)	496 (62.2)	.48	502 (62.0)	.79
Donors	70 (86.4)	10 (76.9)	644 (80.8)	.43	654 (80.7)	.21
Recipient–donor sex						
Sex matched	48 (59.2)	7 (53.8)	505 (63.4)		512 (63.2)	
Male/female	6 (7.4)	1 (7.7)	72 (9.0)	.72	73 (9.0)	.52
Female/male	27 (33.4)	5 (38.5)	220 (27.6)		225 (27.8)	
Disease						
AML	19 (23.4)	2 (15.4)	203 (25.5)		205 (25.3)	
ALL	6 (7.4)	5 (38.4)	153 (19.2)		158 (19.5)	
CML	14 (17.3)	2 (15.4)	101 (12.7)	.11	103 (12.7)	*.02*
MDS	20 (24.7)	2 (15.4)	131 (16.4)		133 (16.4)	
NHL	11 (13.6)	0	88 (11.0)		88 (10.9)	
Other	11 (13.6)	2 (15.4)	121 (15.2)		123 (15.2)	
CMV serostatus						
Donor						
Negative	67 (82.7)	8 (61.5)	600 (75.3)		608 (75.1)	
Positive	14 (17.3)	5 (38.5)	196 (24.6)	.16	201 (24.8)	.13
Missing	0	0	1 (0.1)		1 (0.1)	
Recipient						
Negative	52 (64.2)	8 (61.5)	472 (59.2)		480 (59.4)	
Positive	26 (32.1)	5 (38.5)	297 (37.3)	.66	302 (37.2)	.36
Missing	3 (3.7)	0	28 (3.5)		28 (3.4)	
Recipient–donor CMV status	51 (63.0)	6 (46.2)	415 (52.1)			
Negative/negative	1 (1.2)	2 (15.4)	56 (7.0)	.22	421 (52.0)	
Negative/positive	13 (16.0)	2 (15.4)	164 (20.6)		58 (7.2)	.10
Positive/negative	13 (16.0)	3 (23.0)	133 (16.7)		166 (20.5)	
Positive/positive	3 (3.8)	0	29 (3.6)		136 (16.8)	
Missing				.13	29 (3.6)	
————						
Matched	64 (82.1)	9 (69.2)	548 (71.4)		557 (71.3)	
Mismatched	14 (17.9)	4 (30.8)	220 (28.6)		224 (28.7)	*.04*
Disease risk: European Society for Blood and Marrow Transplantation score						
Good	41 (50.6)	5 (38.5)	360 (45.2)		365 (45.1)	
Intermediate	30 (37.0)	5 (38.5)	290 (36.4)	.69	295 (36.4)	.34
Poor	8 (9.9)	2 (15.4)	125 (15.7)		127(15.7)	
Missing	2 (2.5)	1 (7.6)	22 (2.7)		23 (2.8)	
Stem cell source						
Bone marrow	35 (43.2)	7 (53.8)	360 (45.1)		367 (45.3)	
Peripheral blood stem cell	46 (56.8)	6 (46.2)	430 (53.9)	.76	436 (53.8)	.67
Missing	—	—	7 (.9)		7 (.9)	
Conditioning regimen						
Myeloablative	34 (42.0)	11 (84.6)	412 (51.7)		423 (52.2)	
Reduced intensity	47 (58.0)	2 (15.4)	366 (45.9)	*.011*	368 (45.4)	.05
Missing	0	0	19 (2.4)		19 (2.3)	
T cell depletion (Campath)						
Yes	66 (81.5)	9 (69.0)	650 (81.0)		659 (81.3)	
No	4 (5.0)	1 (8.0)	45 (6.0)	.87	46 (5.7)	.79
Missing	11 (13.5)	3 (23.0)	102 (13.0)		105 (13.0)	
Era						
1996-1999	10 (12.3)	1 (7.7)	86 (10.8)		87 (10.7)	
2000-2003	21 (25.9)	4 (30.8)	284 (35.6)	.41	288 (35.6)	.13
2004-2007	22 (27.2)	4 (30.8)	237 (29.7)		241 (29.8)	
2008-2011	28 (34.6)	4 (30.8)	190 (23.8)		194 (24.0)	
Previous autografts						
0	71 (87.7)	12 (92.3)	677 (84.9)	.62	689 (85.1)	.53
≥1	10 (12.3)	1 (7.7)	120 (15.1)		121 (14.9)	

Values are n (%) unless otherwise defined. AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; CDS, coding DNA sequence.
Numbers in italics represent significant p values (<0.05).

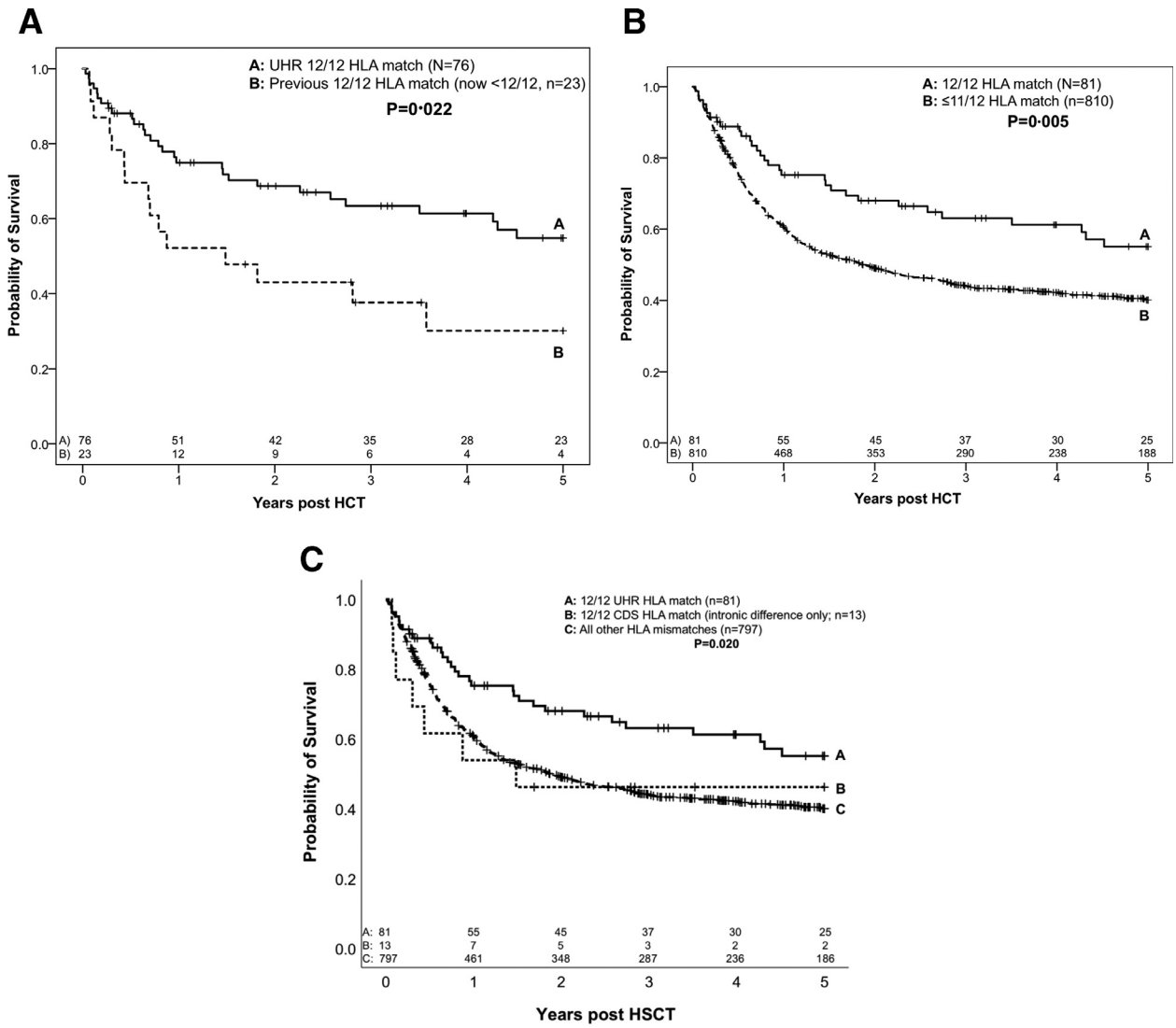

Figure 1. The impact of UHR HLA matching status as achieved with NGS on OS. (A) A comparison of previously identified 12/12-matched patients when stratified into those who remain 12/12 HLA matched (n = 76) and those found to be HLA mismatched (n = 23). (B) A comparison of UHR 12/12 HLA-matched patients (n = 81; includes 76 patients previously believed to be 12/12 matched and an additional 5 pairs previously believed to be less well matched but identified as 12/12 matched after UHR HLA typing) and those with any degree of HLA mismatching as identified by NGS (n = 810). (C) The impact of noncoding variation on transplant outcome. The 12/12 UHR HLA-matched pairs have significantly higher survival probabilities than patients who received either a 12/12 CDS match (ie, where there is the presence of only intronic or untranslated region mismatches) or any other HLA mismatch.

The numbers above the *x*-axis denote the number of patients at risk at each time point.

had significantly higher 5-year OS (55.1%; 95% CI, 43 to 67) than those with any degree of mismatching (n = 810; 40.1%; 95% CI, 37 to 44; *P* = .005) (Figure 1B).

Impact of Noncoding Variation on HCT Outcome

HLA mismatched pairs (n = 810) were divided into those where the mismatches only differed in the introns and untranslated regions of the HLA genes, and thus were 12/12 CDS HLA matches, and those where coding mismatches were observed (all other mismatches) (Figure 1C). The number of pairs with only noncoding differences were low (n = 13). Five-year OS was significantly higher in 12/12 UHR HLA-matched patients (n = 81; 55.1%; 95% CI, 43 to 67) than in those receiving either a 12/12 CDS HLA match (n = 13; 46.2%; 95% CI, 23 to 71) or any other mismatched donor (n = 797; 40.0%; 95% CI, 37 to 44; *P* = .02). Unfortunately, the number of patients who were only mismatched in non-ARD exons (ie. matched for the ARD, introns, and untranslated regions) were too low to allow for outcome analysis to be performed (n = 3).

Implementing HLA-DPB1 TCE Models

Patients identified as 10/10 UHR HLA matched (n = 523) were coded as being 12/12 HLA matched (n = 81), permissively mismatched for their DP-TCE (TCEM; n = 231), or nonpermissively mismatched (TCED; n = 211) [11] (for details on how TCE matching status changed with UHR HLA typing, see Supplementary Table S3). Individuals with a 12/12 UHR HLA match had the best survival (5-year OS: 55.1%; 95% CI, 43 to 67), whereas the 10/10 UHR-matched TCED group did significantly worse (31.5%; 95% CI, 25 to 39; $P < .0001$) (Figure 2A). There

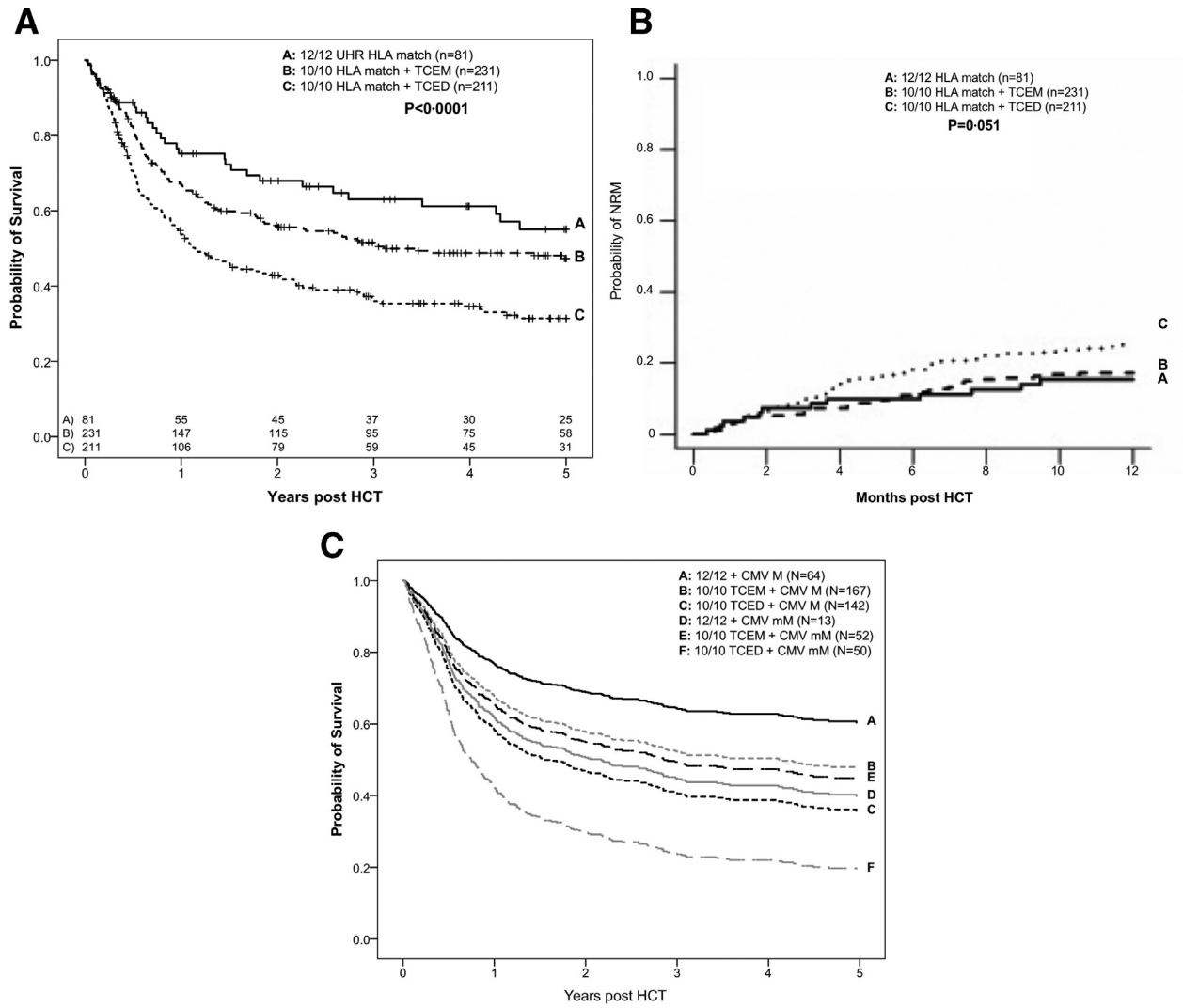

Figure 2. The impact of UHR HLA typing and HLA-DPB1 TCE matching on transplant outcome. (A) The 12/12 UHR HLA-matched patients have significantly higher OS than 10/10 TCEM and 10/10 TCED patients. (B) Inferior survival is due to increased probabilities of NRM. (C) Adjusted curves showing UHR HLA matching and CMV matching on OS. The numbers above the *x*-axis denote the number of patients at risk at each time point.

was no significant difference in OS between 12/12 and 10/10 UHR TCEM pairs ($P = .13$).

The probability of 1-year NRM for the whole cohort was 20.4% (95% CI, 17 to 24), whereas grades II to IV aGVHD was observed in 27.7% of cases. Nonpermissive DP-TCE mismatching was also correlated with an increase in 1-year NRM (Figure 2B). Recipients receiving a 10/10 TCED graft had a trend for higher NRM than 10/10 TCEM or 12/12 matched patients (25.7% [95% CI, 20 to 33], 17.3% [95% CI, 13 to 23], and 16.8% [95% CI, 9 to 26], respectively; $P = .051$). An increased risk of grades II to IV aGVHD was observed with increasing levels of HLA mismatching. Patients receiving a 12/12 UHR HLA-matched graft had the lowest risk of grades II to IV aGVHD (15.8%), whereas an almost 2-fold increase was associated with DP-TCE mismatching (26.4% and 29.4% for TCEM and TCED patients, respectively; $P = .068$). No significant differences in disease relapse between the 3 groups were observed ($P = .73$).

When compared with 12/12 HLA-matched patients, the detrimental effect of being 10/10 UHR HLA-matched TCED persisted in multivariate analysis of OS (hazard ratio, 1.98; 95% CI, 1.3 to 2.9; $P = .001$) (Table 2). Covariables included in this analysis were patient age, transplantation era, European Society for Blood and Marrow Transplantation risk score, and recipient–donor CMV matching status. Nonpermissive DP-TCE mismatching was correlated with a trend for increased risks of NRM (hazard ratio, 1.75; 95% CI, .92 to 3.3; $P = .091$) and significantly higher risks of grades II to IV aGVHD (odds ratio, 2.37; 95% CI, 1.1 to 4.9; $P = .018$) when compared with 12/12 HLA-matched patients. A trend for increased aGVHD was observed for patients with a TCEM donor (relative risk, 1.98; 95% CI, .97 to 4.0; $P = .059$). Covariables for these analyses included patient age and reduced-intensity conditioning (NRM only), era (NRM and aGVHD), and donor age (aGVHD).

Impact of UHR HLA Typing and CMV Matching

Because we have previously shown the beneficial impact of considering both HLA and CMV matching on UD-HCT outcome [6], we investigated the impact of CMV matching when UHR HLA matching was considered. Significant differences in 5-year survival probabilities were observed with the best outcome in 12/12 UHR HLA-matched, CMV-matched patients (group A: 62.0%; 95% CI, 48 to 74) and worst in 10/10 UHR HLA-matched, TCED, CMV-mismatched patients (group F: 17.5%; 95% CI, 9 to

Table 2
Multivariate Analysis of OS, NRM, and aGVHD

	OS			NRM			aGVHD Grades II-IV		
	No. of Cases	Relative Risk (95% CI)	*P*	No. of Cases	Relative Risk (95% CI)	*P*	No. of Cases	Odds Ratio (95% CI)	*P*
HLA matching status									
12/12	76	1.0		77	1.0		76	1.0	
10/10 + TCEM	219	1.28 (.8-1.9)	.24	221	1.13 (.6-2.2)	.72	159	1.98 (.97-4.0)	.059
10/10 + TCED	192	1.98 (1.3-2.9)	.001	201	1.75 (.92-3.3)	.091	155	2.37 (1.1-4.9)	.018
Patient age									
<40 yr	217	1.0		224	1.0		—	—	—
>40 yr	270	1.43 (1.09-1.8)	.009	275	1.83 (1.1-3.1)	.019			
Era									
1996-1999	38	1.0		36	1.0		34	1.0	
2000-2003	161	1.15 (.6-1.9)	.57	158	.85 (.4-1.9)	.69	159	.35 (.16-.79)	.011
2004-2007	140	.79 (.4-1.3)	.39	159	.75 (.4-1.7)	.49	154	.43 (.19-.97)	.041
2008-2011	148	.89 (.5-1.5)	.69	146	.87 (.4-2.0)	.74	143	.39 (.17-.93)	.033
European Society for Blood and Marrow Transplantation risk score									
Good	230	1.0							
Intermediate	183	1.38 (1.0-1.8)	.018	—	—	—	—	—	—
Poor	74	1.29 (.8-1.8)	.17						
Patient–donor CMV matching status									
Matched	372	1.0		—	—	—	—	—	—
Mismatched	115	1.38 (1.05-1.8)	.019						
Conditioning									
Myeloablative	—	—	—	241	1.0		234	1.0	
Reduced intensity				258	.61 (.4-.98)	.036	257	.64 (.4-.98)	.044
Donor age									
<30 yr	—	—	—	—	—	—	167	1.0	
>30 yr							324	1.75 (1.1-2.8)	**.018**

Bold type was used to denote significant P values (<0.05).

31; overall $P < .001$). These correlations persisted after adjustment in multivariate analysis. The greatest associations were observed between 12/12 HLA-matched, CMV-matched patients (group A) and either 10/10 HLA-matched, TCED, CMV-matched (group C) or CMV-mismatched (group F) patients. When compared with the use of a group A donor, patients had a significantly increased risk of mortality with a group C (relative risk, 2.03; 95% CI, 1.02 to 3.30; $P = .004$) or a group F donor (relative risk, 3.25; 95% CI, 1.90 to 5.55; $P < .0001$) (Figure 2C).

DISCUSSION

NGS and third-generation sequencing technologies have changed the landscape for HLA typing laboratories, allowing for full gene characterization in a manner that is viable for use in a routine clinical typing laboratory, and thus is becoming current practice. Differences between the technologies are minimal. Both technologies allow for more of the HLA gene to be sequenced, with introns and untranslated regions included routinely. NGS technologies are limited by short read lengths, resulting in ambiguous HLA typing data. For example, any 2 alleles that differ only at regions further apart than the maximum read length of the technology cannot be phased and will result in HLA typing ambiguity or miscalled alleles. Conversely, third-generation sequencing technologies have the ability to generate long reads sequenced in isolation and thus generate fully phased sequences that reduce the potential for ambiguity and/or miscalled alleles. In our opinion the current optimal typing technology is Pacific Biosciences Single Molecule Real-Time DNA sequencing, which has the ability to sequence individual molecules of double-stranded DNA of up to 10 kilobase pairs at the high level of accuracy required for HLA typing

This study shows for the first time that polymorphism in previously uncharacterized regions of the classic HLA genes affects VUD-HCT outcomes. HLA matching at UHR as achieved by single-molecule real-time sequencing results in superior OS because of reduced NRM and aGVHD risks. Additionally, we confirm our previous findings that HLA and CMV matching can be used together to further refine the prediction of outcome risks.

At the start of this project we had hoped to identify the impact of noncoding variation and mismatching of non-ARD exons on VUD-HCT outcome; however, we identified limited numbers of pairs with each of these types of mismatch. The number of patients who were mismatched at a noncoding level only were too low for impactful clinical analysis in this cohort, but it was observed that outcomes were similar for CDS HLA-mismatched pairs and those with any other UHR HLA mismatch. Further studies on larger cohorts of individuals are needed to confirm our findings and to enable more complex analyses that may help elucidate if there are differences in patient outcomes when 12/12 UHR HLA-matched, 12/12 CDS-matched, or UHR ARD-matched donors are used.

A possible explanation for the significant survival benefit conferred by a 12/12 UHR HLA match is that typing methods that consider the additional regions of the gene are acting as markers for demonstrating haplotype compatibility. The beneficial impact of MHC haplotype matching on the outcome of VUD-HCT has been shown previously [25,26]. In these studies MHC haplotypes were determined either by physical separation using a probe-based DNA capture method and resequencing of the HLA genes at medium/high resolution or inferred by the screening of a panel of MHC-located, non-HLA single nucleotide polymorphisms. Both studies showed increased GVHD with haplotype mismatching, although the impact on OS was negligible. We suggest that matching at a 12/12 UHR level is a step toward achieving a degree of compatibility closer to an MHC haplotype match and thus may be associated with improved survival prognoses, as observed in this study. Further studies are required to test this hypothesis.

Several studies have shown an increased risk of disease relapse with increased levels of matching between VUD-HCT recipients and donors, possibly because a degree of genetic

disparity is required to initiate graft-versus-leukemia responses [8,9,12,27-29]. In particular, recipients of MHC haplotype matched transplants were identified as having a significantly reduced risk of grades III to IV aGVHD but also a significantly higher risk of disease relapse [25]. Surprisingly no significant differences in relapse risk were observed between the 3 groups in this study ($P = .73$). It is possible that although allele and haplotype matching was inferred in the VUD-HCT pairs included in the MHC haplotype analysis studies from medium-/high-resolution HLA typing data, the level of matching achieved with UHR analysis of these HLA types would have revealed additional differences that suggested the definition of "matched" was inaccurate. The data from this study may suggest that true MHC haplotype matching, as defined by UHR HLA typing, may achieve a balance of reducing GVHD responses but retaining the graft-versus-leukemia effect, although this requires confirmation in additional data sets.

This study confirms the significant impact of HLA-DPB1 matching on VUD-HCT outcome. The model of DPB1 matching described in this study has been shown to improve outcome previously, with 12/12 HLA-matched and 10/10 TCEM patients having better OS and NRM in a large cohort of VUD-HCT pairs [8]. Although outcome prognoses were significantly improved in this earlier study, the difference in risk between the 3 groups was low (hazard ratios of 1.0, .96, and 1.15 for 10/10 TCEM, 12/12 matched, and 10/10 TCED patients, respectively), which is in contrast to the differences described in our study (hazard ratios of 1.28, 1.0, and 1.98 for the 3 groups, respectively; overall 15% difference in OS at 5 years between the 3 groups). Reanalysis of the 891 patients included in this study according to this HLA/DP-TCE model using our original intermediate-/high-resolution HLA typing data demonstrated OS probabilities similar to those reported by Fleischhauer et al. [8] (data not shown). The larger differences observed with UHR HLA typing suggest that this level of typing may provide more power to discriminate between DP-TCE groups, could help to further refine the DP-TCE algorithms, and strengthen the argument for including HLA-DPB1 in VUD selection strategies.

The data presented here also build on our previous findings that HLA and CMV matching should be jointly considered when selecting VUDs [6]. The patients included in this analysis were all defined as UHR 10/10 HLA matches, which according to the current gold standard is the best possible VUD option for patients. However, we were able to demonstrate up to 45% difference in OS probabilities with the inclusion of CMV serologic data. As with DP-TCE matching, the use of UHR HLA matching combined with CMV matching status allowed a more refined stratification of donors and could provide a novel matching model for VUD-HCT. Because patients in this cohort were transplanted before the recent move toward using younger VUDs, we were unable to include donor age into our analyses, but we hypothesize that survival probabilities will be additionally affected by the use of younger donors [2,6,13].

A limitation of the typing strategy used here is that full gene sequencing was not performed for HLA class II genes. The decision to use an extended sequencing protocol that included all exons encoding the extracellular domains of the mature protein was due to the lack of available and reliable reference sequences for the HLA class II genes when establishing the typing and analysis protocols. It will be important to determine the impact of definitive HLA class II allele typing on VUD-HCT outcome. We hypothesize that matching at a definitive HLA class I and II allele level will result in further survival advantages, possibly because of the ability to further predict MHC haplotype matches. A full-length HLA class II typing strategy is in development and once completed will be used to retype and reanalyze this cohort [30].

It will be important to consider how UHR HLA typing affects the outcome of VUD-HCTs that include other methods of T cell depletion and T cell–replete transplants. Additionally, we did not have sufficient 9/10 HLA-matched pairs to determine the impact of UHR HLA typing and DP-TCE matching on their outcome prognoses. Alternative means of determining permissive HLA-DPB1 mismatching according to expression levels have been proposed [31] but are not tested here to avoid further complicating these matching algorithms. Future studies will endeavor to determine the impact of HLA-DPB1 expression status on this cohort. A final limitation of this study is the absence of chronic GVHD analyses, resulting from limited patient outcome reporting. Further and larger studies are required to elucidate these observations.

In conclusion, our study demonstrates for the first time the importance of UHR HLA typing as achieved with NGS on the outcome of VUD-HCT. Based on these findings, we propose that 12/12 UHR HLA-matched, CMV-matched donors be preferentially selected for recipients with a hematologic malignancy undergoing VUD-HCT and that HLA-DPB1 TCED donors should be avoided where possible. The benefit of this approach could be particularly advantageous for patients with a choice of many donors. At this time we are unable to make recommendations on donor selection for patients $\leq$9/10 UHR-matched donor; in such cases we would suggest that time to transplant should be considered and that current guidelines for donor selection should be followed. We also suggest that increasing the number of donors selected at the point of confirmatory or verification typing will consequently increase the probability of identifying 12/12 or 10/10 TCEM donors. Although this will increase the initial costs of VUD-HCT, the reduced risk of post-transplant complications coupled with the potential for improved patient well-being should not be overlooked. The onus is now on unrelated donor registries to provide UHR HLA typing on their donors prospectively at the time of donor search and that clinical HLA typing laboratories should be able to type and match patients and donors at this level of resolution, thus becoming the new standard of care for patients.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.12.768.

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